REMARKS

Applicants appreciate the thorough examination of the present application as evidenced by the Office Action mailed July 15, 2003.

Claims 1-14 are pending in the present application. Claims 1-13 stand rejected. Claim 14 has been objected to in the Office Action. The concerns raised by the Examiner are addressed below.

I. New Claims

Applicants have added new claims 15-19. Support for new claims 15-17 can be found at pages 4-7 of the specification, among other places. Support for new claims 18 and 19 can be found at pages 1-3, 6 and 7 of the specification, among other places. Accordingly, the Applicant respectfully submits that these new claims are fully supported by the application as filed, and respectfully request entry and examination thereof.

II. Claim Objections

Claim 14 is objected to under 37 C.F.R. § 1.75(c) as being in improper form. Applicants have amended claim 14 to dependent from independent claim 1. Accordingly, Applicants respectfully request that this objection be withdrawn.

III. Claim Rejections Under 35 U.S.C. § 103

Claims 1-13 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 96/05846 to Nebe (Nebe), U.S. Patent No. 5,696,236 to Omar et al. (Omar et al.) and EP 0798003A2 to Savage et al. (Savage et al.). More specifically, the Office Action relies upon the following statements in support of the obviousness rejection of the present invention over the combination of Nebe, Omar et al. and Savage et al.:

> It would have been obvious to one of ordinary skill in the art to utilize a depth filter, which is ordinarily used in the art as a prefilter for ultramembrane filtration, for the removal of prion particles from a liquid based on the observation of Nebe which indicated that **half of the infectious prion was removed using the nylon premembrane filter (depth filter)** indicating that the prion has a high expectation of success utilizing the matrices of Omar et al. for the removal of infectious agent from blood plasma products.

Office Action, page 4 (citations omitted) (emphasis added). Applicants respectfully traverse this rejection.

A depth filter is a specific type of filter that is entirely different from the membrane filters referred to in Nebe. Depth filters generally refer to a filter medium consisting of randomly distributed particles or fibers resulting in openings with a non-uniform and tortuous path. *See* Tab 1 presenting a schematic showing a comparison of depth filters, microporous filters and molecular membrane filters and technical information regarding Seitz® depth filters. In contrast, the gauge and microporous membranes used by Nebe can be characterized as "pre-filters" (and more surface-type filters) to provide decreasing pore size and are used in this manner to prevent the subsequent ultrafiltration membranes from being contaminated with microbes during the ultrafiltration processing steps. Thus, the "pre-filters" utilized in Nebe are not "depth filters" and, thus, are not equivalent to the filters employed in the methods of the present invention.

Moreover, the filters employed by Nebe were **not effective** in removing prion proteins, where nearly five logs of infectivity/ml passed through the filters as shown on page 13 of Nebe. Applicants provide herewith at Tab 2, an unofficial translation of portions of Nebe for the purpose of responding to the Office Action. A review of the data indicates that the titre of the infectious scrapie material was $10^{8.45}$ LD50/ml. *See* unofficial translation of Nebe, Example 1, Section 1.3, last paragraph). 10 ml of the scrapie spike was added to 600 ml of the thymus homogenate (*see* unofficial translation of Nebe, Example 1, Section 1.3, first paragraph) which provided a starting level of infectivity of $10^{6.66}$ LD50/ml. Figure 1 of Nebe, right of the flow chart, shows a "reduction" in infectivity of only $10^{1.23}$. Thus, the final infectivity is approximately $10^{5.4}$. This correlates approximately with the figure of $10^{4.97}$ given as the infectivity of Probe A. Consequently, there is a minimal reduction in infectivity of 1.23 logs/ml leaving a high residual infectivity of about 5 logs/ml.

In contrast, the examples of the present application show that there is **no detectable prion protein in the filtrate after filtration** according to the methods of the present invention. The examples further show that other types of filters, such as those employed in some of the cited references, were not effective in removing prion proteins. Results are summarized in Table 1 on page 14 of the specification and included herewith at Tab 3 for the Examiner's convenient reference. Specifically considering Nebe at Example 1, Section 1.1 (*see* unofficial translation of Nebe), Nebe utilizes a crude brain extract to spike the solution with prions. The crude brain extract can contain infected cellular material and tissues which can be removed by nylon gauze and membrane filters. However, soluble and/or highly

dispersed prions that remain in solution after the removal of larger molecular size material are not readily removed. The larger materials are removed on the basis of size alone by sieving out the large clumps. **Nebe failed to achieve removal of the soluble/dispersed material as five logs of prion material passed through the combination of gauze and membrane filters**. According to embodiments of the present invention, the soluble and/or highly dispersed prion proteins remaining in solution can be removed by the methods of removal recited in the claims of the present invention.

It is clear that Nebe does not teach or suggest prion removal comprising passing a protein-containing solution through a filter comprising a matrix having a solid particle comprising a porous material and a pore size of not greater than 6 μm, wherein the protein is an abnormal infective prion protein as recited in the claims of the present application. In fact, Nebe fails to provide prion removal to any level that is useful where such levels shown in Nebe could present a public concern.

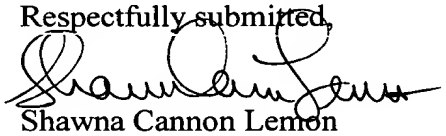
Applicants respectfully submit that neither Savage et al. nor Omar et al. cure the deficiencies of Nebe. Savage et al. relates to the use of structured depth filters for virus removal. Omar et al. relates to a method for the removal of viruses from protein solutions. At the time of filing the present application, the scientific community had concluded that prion proteins are not viruses. In fact, prions can be easily distinguished from viruses. Thus, problems associated with clearance of viruses from aqueous solutions are distinct from problems associated with clearance of prion proteins from aqueous solutions, in particular, abnormal infective prion proteins which present a distinct conformation as compared to native prion proteins. As such, one of ordinary skill in the art would not rely upon Savage et al. or Omar et al. which discuss methods of virus removal in order to address the problems associated with removal of abnormal infective prion proteins.

For the reasons set forth above, Applicants respectfully submit that one of ordinary skill in the art would not rely upon Nebe alone, or in combination with Savage et al. and Omar et al., in order to arrive at the present invention relating to removal of abnormal infective prion proteins as recited in the claims of the present application. Accordingly, Applicants respectfully submit that claims 1-14 and newly added claims 15-19 are not unpatentable under 35 U.S.C. § 103(a) in view of Nebe, Omar et al. and Savage et al., and respectfully request that this rejection be withdrawn.

Conclusion

In view of the foregoing amendments and remarks, Applicants respectfully request that all outstanding rejections to the claims be withdrawn and that a Notice of Allowance be issued in due course. The Examiner is invited and encouraged to contact the undersigned directly if such contact will expedite the prosecution of the pending claims to issue. In any event, any questions that the Examiner may have should be directed to the undersigned, who may be reached at (919) 854-1400.

Respectfully submitted,



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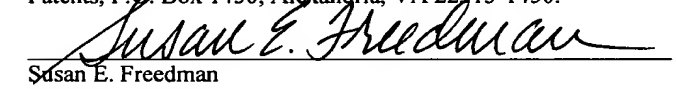
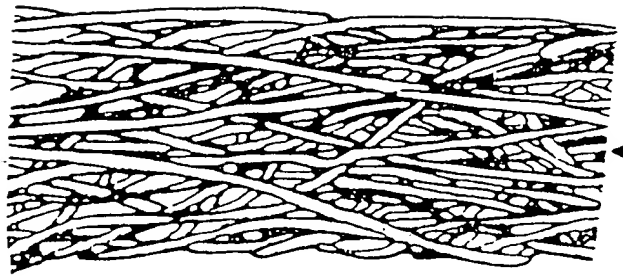
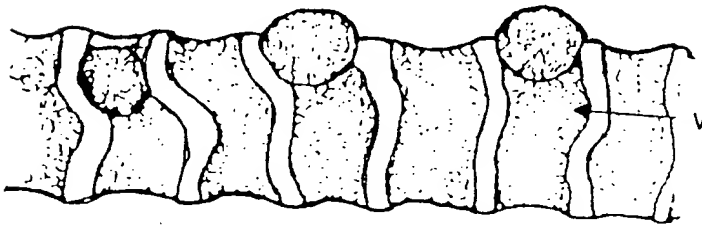


Susan E. Freedman
Date of Signature: November 17, 2003

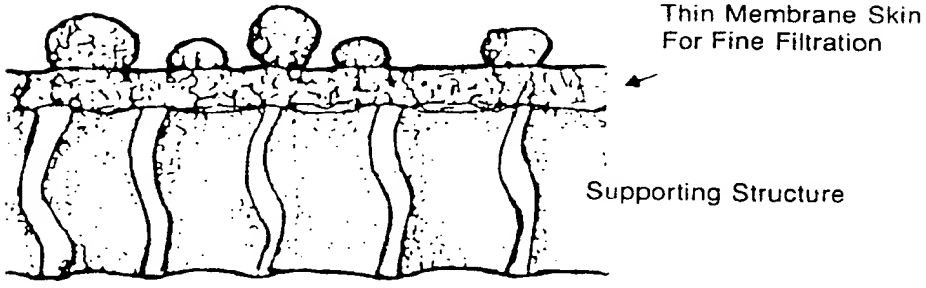
Filter Types



Depth Filters



Microporous Filters



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SEITZ depth filters

the technological lead

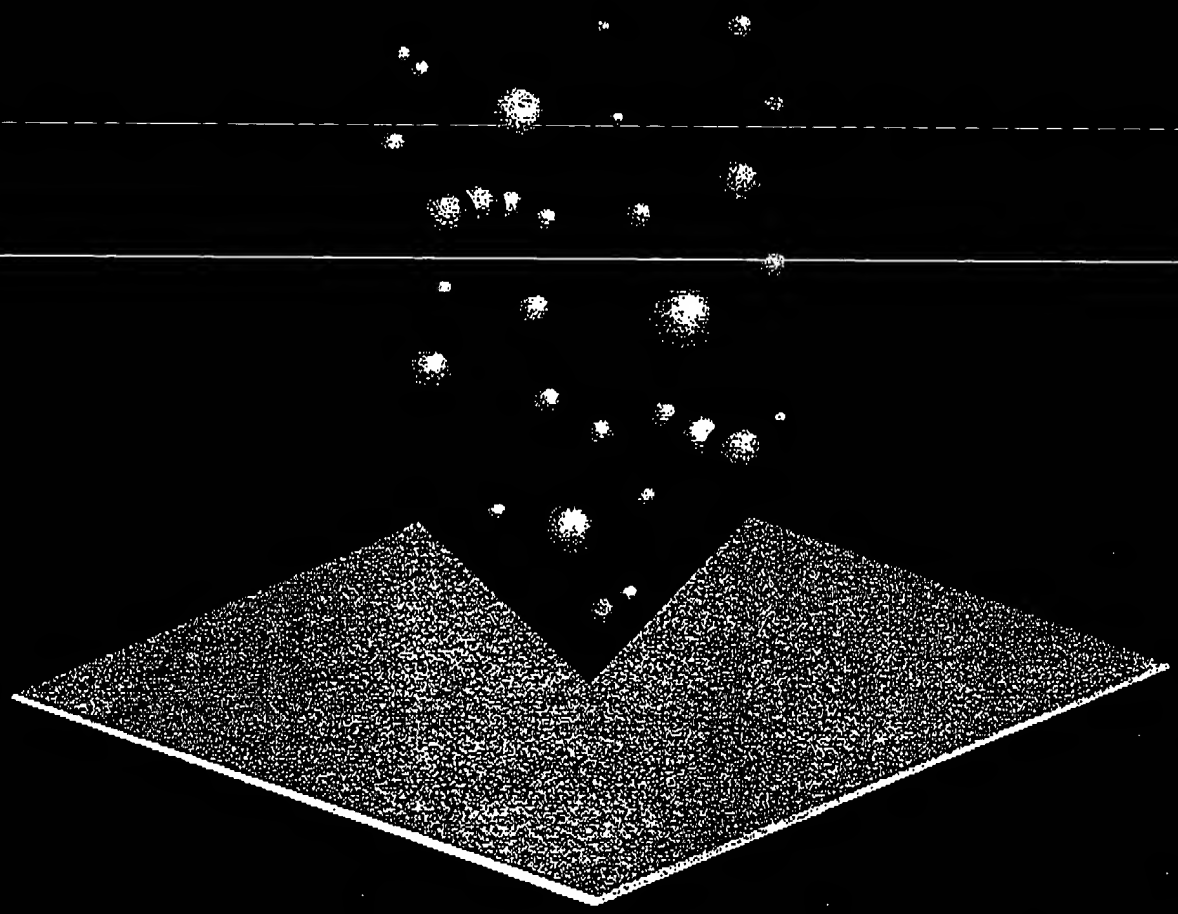

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Table of contents

SEITZ filtration technology: without alternative when considering all the advantages

The technological lead has a name: SEITZ

The first effective microfilter was marketed in 1914. Developed and produced by SEITZ. Ever since all important progress in depth filtration technology is closely linked with the name SEITZ. SEITZ realized dependable depth filtration without asbestos.
Last but not least, continuous striving for technical progress and even better quality have advanced the brand SEITZ to its present high esteem.

SEITZ depth filters, the challenge in comparison

It is worth-while to compare the present range of SEITZ depth filters with those which you have used for many years to solve numerous process-technological problems.
You will rediscover all the positive aspects which have always been characteristic for SEITZ. The high degree of reliability, their efficiency, the economical advantages and simple handling. And, of course, the typical, uniform product quality.
None the less the present grades of SEITZ depth filters represent completely new developments. A thorough study of the retention mechanisms, important in the field of depth filtration, led to the fact that depth filters were virtually newly invented. The result is a filter medium equal to asbestos-containing filter media regarding performance and efficiency and thus a significant progress in depth filtration.

A comparison should convince you.

Low-pollution filtration: SEITZ spares you and the environment a lot

SEITZ has achieved the impossible: the development of non-asbestos depth filters with equal or, in part, even better properties than those made with asbestos fibres.
In close cooperation with numerous users in the most varied industries, field tests were repeatedly carried out with the objective to establish that the newly developed filters meet the requirement profile. In this context we would like to thank our clients.
The development of non-asbestos depth filters progressed through several phases until the present state of art was achieved. Initially, very fine grades of kieselguhr were used in a tight cellulose matrix.

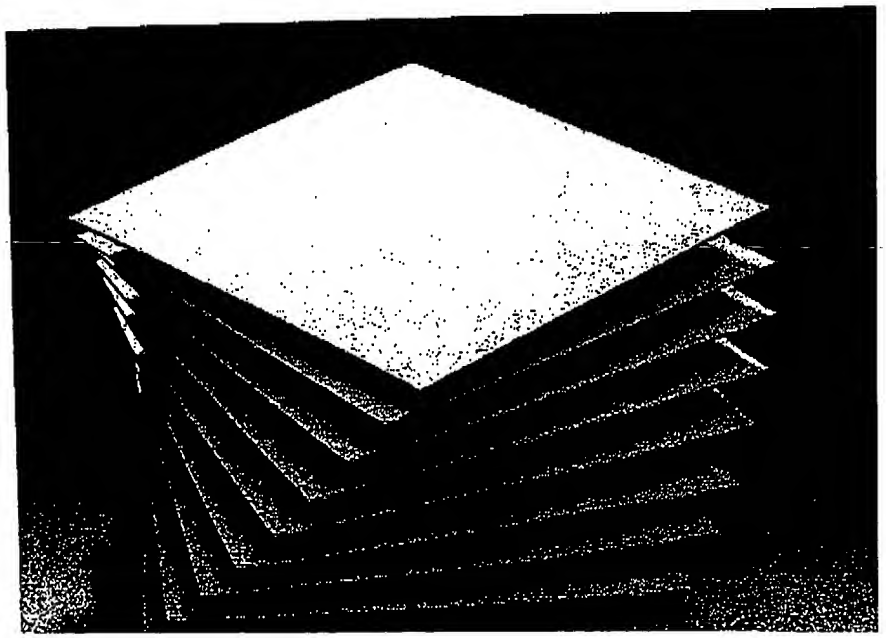
Result:
high edge leakage rates in filter units and premature blocking in case of media with a high challenge.

Other developments were directed towards the addition of agents carrying a charge with a view of substituting asbestos with a high zeta potential.

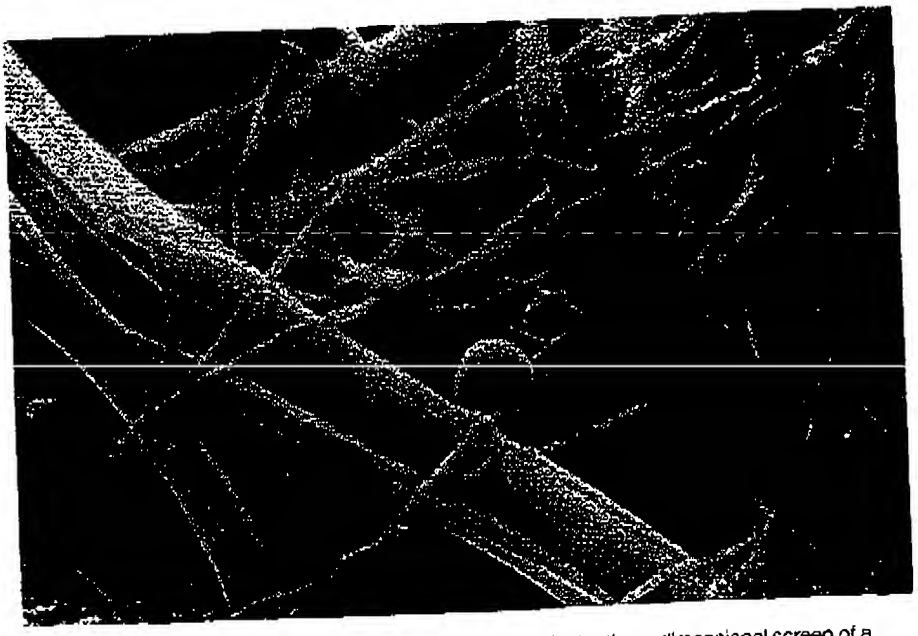
Result:
break-through in case of flow fluctuations and excessive retention of colour components/effective substances respectively colloids, causing rapid blockage.

All this is history, the technical state o art has since been newly defined by SEITZ.

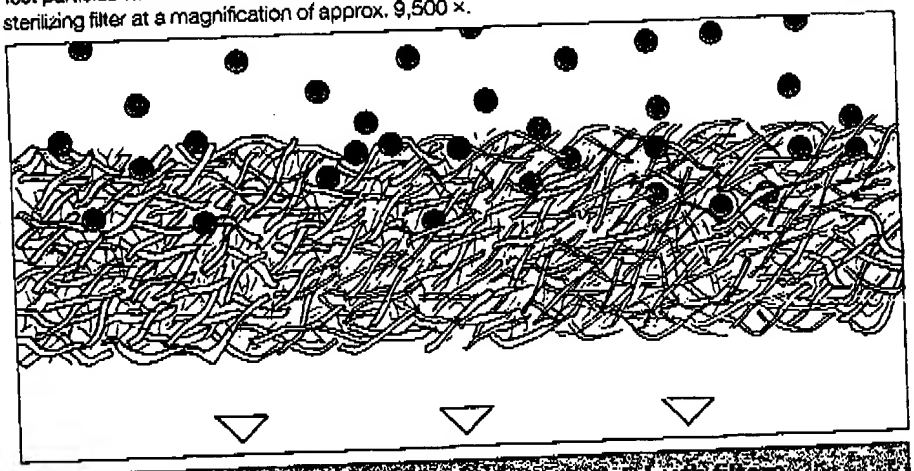
The solution to the problem "asbestos substitution" can be summarized as follows: in elaborate test series involving a large variety of raw materials a very finely balanced optimal relationship was established between specia cellulose upgrading procedures permitting the use of fine kieselguhr with out high edge leakages, precisely dosed charge carriers and further SEITZ-specific parameters within the manufacturing process.



The mechanisms of depth filtration



Test particles with a diameter of 1.05 and 1.45 µm trapped in the three-dimensional screen of a sterilizing filter at a magnification of approx. 9,500 ×.



Factors affecting the retention rates of depth filters

Mechanical factors		Adsorptive factors	
Product	**Depth filter**	**Product**	**Depth filter**
● Nature of the impurities/particles ● Number and size of the impurities/particles ● Viscosity Chemical composition	● Void volume of the filter medium ● Structure of the three-dimensional screen Size of the internal surface area (dirt holding capacity) ● Thickness of the filter medium Nature, upgrading and composition of the individual material components	● Chemical composition ● Charge of the impurities/particles Concentration of the impurities/particles pH-value ● Flow velocity ● Polarity of the impurities/particles ● Temperature	● Structure of the three-dimensional screen ● Number of the charge carriers ● Nature of the charge carriers Magnitude of the charge Polarity of the charge carriers

In the various SEITZ laboratories extensive analyses of the complex depth filtration mechanisms were carried out. Due to their material composition and their structural design SEITZ depth filters can basically be compared with a maze-like, extremely fine three-dimensional sieve with innumerous, branched micro "channels". They form a structure with a void volume of 70–85 % of the total volume of the depth filter. This is indicative of the high dirt holding capacity. The void volume of a SEITZ depth filter can amount to as much as 4 L per m^2 filter area.

Passage of the liquid through the innumerable channels is relatively slow so that the contact time with the filter medium is relatively long. Particles, microorganisms, colloids, virus and pyrogens are trapped on their long passage through this fine maze with a synergistic effect between the three-dimensional mechanical screen and the adsorptive capabilities of the electrokinetic potential.

The table on the left indicates the factors which influence the retention rates of SEITZ depth filters.

To convey a vivid image of the proportions within a depth filter, the following comparison may be helpful: If a particle with a size of 1 µm requires about 34 seconds for its passage through a 3.7 mm thick filter at realistic filtration conditions (400 L m^{-2} h^{-1}/ 0,11 m s^{-1}) analogously a football would have to travel in the same time through an approx. 87 m deep, dense forest interspersed with brushwood without becoming trapped.

Nebe WO 96/05846
translation (partial).

Example 1

Complete removal of scrapie virus. As a model for so called slow viruses or prions, scrapie virus was bred in a group of hamsters and after spiking administered by 5 serial cartridges on a production typical ultrafiltration plant and the infectivity of the ultrafiltrates examined in an animal test (see figure 1).

1.1 *Obtaining infectious scrapie virus*

A hamster brain infected with scrapie stock+ 263K was macerated and pooled in terminal stage. 500mg of this was added to 4.5ml sterile physiological salt dilution, homogenised and centrifuged for 10 min at 500g. 50µl of the remainder was injected intracerebrally into 40 Syrian LVG-hamsters. The animals were euthanised after this has become necessary due do their clinical state. This took on average 65 to 80 days from the injection. The brains were removed aseptically, frozen, macerated, mixed with a sterile cooking salt dilution and homogenised. Afterwards, they were centrifuged for 10min at 500g and the remainder was used as "spike" material.

1.2 *Obtaining thymus gland homogenate*

Three calves` thymus glands (ca. 550g) were pureed, mixed with 1.5L pyrogen free water and homogenised. The homogenate was stored at 4°C until usage.

The pre-filters and the ultrafilters were washed with water, as usual. For the pre-filtration, thymus extract was filtered through nylon gauze and three microfiltration membranes (Pall, nylon membranes) with pore sizes 2.0µm, 0.8µm and 0.2µm. To ensure that the virus` distance was not falsified through particle absorption on the large inner surface of the spiral cartridges and to measure filtration reduction only, filters were pre-spiked for virus reduction. Pre-filters and filters were pre-treated with the thymus homogenate obtained, i.e. only a small amount (4x 200ml) was passed through the filter at a time.

1.3 *Removal of the scrapie virus*

10ml of the scrapie spike dilution obtained in sample 1 was added to 600ml of the thymus homogenate and stored overnight at 4°C. Then, the dilution was filtered through a first pre-filter with a pore size of 2.0µm, a second pre-filter with a pore size of 0.8µm and a third pre-filter with a pore size of 0.2µm. The filtrate was labelled as Probe A.

190ml of the spiked pre-filtrate material was again mixed with 6ml of the scrapie spike dilution and then ultrafiltered with an Amicon membrane S1Y30. The filtrate was labelled as Probe B.

144ml of Probe B was again mixed with 6ml of the scrapie spike material and ultrafiltered for a second time with another Amicon S1Y30 membrane. The filtrate was labelled as Probe C.

121 ml of Probe C was again mixed with 6ml of the scrapie spike material and again ultrafiltered with the same membrane. The filtrate is labelled was Probe D.

121ml of Probe D was ultrafiltered for a fourth time with the same membrane. The dilution obtained was labelled as Probe E.

93ml of Probe E was ultrafiltered for a fifth time with the same membrane. The dilution obtained is labelled as Probe F.

Probe F subsequently was led via a sterile filtration to a 0.2µm filter. The filtrate obtained is labelled as Probe G.

Figure 1 gives an overview over the method used.

This method gave an infection titre of $10^{8,\ 4\ 5}$ LD50/ml for the starting material. After three pre-filtrations for Probe A $10^{4,\ 9\ 7}$ LD50/ml the total number of infectious virus before the first ultrafiltration after respiking was (6ml x $10^{8,\ 4,\ 5}$) $10^{9,\ 2\ 3}$ LD50 units (150ml). After ultrafiltration, Probe B was given only $10^{4,\ 5\ 6}$ LD50 units ($10^{2,\ 3\ 8}$ LD50/ml). This gives a virus reduction of factor $10^{4,\ 6\ 7}$ at the first ultrafiltration.

Reduction at second ultrafiltration

After respiking the Probe (6ml x $10^{8,\ 4\ 5}$) the dilution (127ml) contained $10^{9,\ 2\ 3}$ virus altogether. The infection titre which was found despite another spiking after the second ultrafiltration was smaller than 1 LD50/ml, i.e. there was no more virus found. This gives a titre in 127ml of $<10^2$ LD50. So the reduction in the second ultrafiltration step is larger than $10^{7,\ 1\ 3}$.

As described previously, Probe G was determined. Here, an reduction factor > $10^{7,\ 2\ 8}$ was found.

Figure 2 shows the reduction data of the scrapie virus through serial ultrafiltration. In contrast to the result theoretically expected, a constant or even decreasing reduction as observed with serial filtration of bacteriophage *fr* over the same ultrafiltration membrane S1Y30 (WO 91/12027, table 3), the reduction rate for the scrapie Virus increases after the second filtration step by more than 2 ten potencies.

Through serial ultrafiltration even the reduction of titre through autoclaving or alkali treatment (both considered as secure) can be fallen short of significantly. That way, you can manufacture a drug without danger potential through virus of spongiform encephalopathies. Independent of this, additional pre or post cleaning steps can of course be combined with this method described. This method distinguished by the Virus being removed through ultrafiltration until the product positively is not infectious any more.

The available experiments prove that with a repetitious ultrafiltration the removal of infectious agents like prions or so called "slow viruses", the reduction speed per filtration step increases at least 100 or 1000fold which leads to a rapid acceleration of virus distance and therefore to an infection security of the product.

Patent Claims

1. Method for the manufacture of pharmaceutical products and/or foods made from material probably contaminated with prions, including the secure removal of the infectious agents: prions only are removed by repetitious filtration through an ultrafiltration membrane and/or a series of ultrafiltration membranes.

2. Method according to one of the previous demands: the infectious material is a virus of spongiform encephalophaty.

3. Method according to one of the previous demands: the virus causes scrapie, BSE, Kuru, Gerstmann-Straeussler, Creutzfeld-Jakob and/or Alzheimer.

4. Method according to one of the previous demands: before use, the filtration membrane is pre-treated with the material to be filtered.

5. Method according to one of the previous demands: from the second ultrafiltration, the virus is removed by at least a factor of 10^{-6}, preferably 10^{-7}.

6. Method according to one of the previous demands: the pharmaceutical product contains thymus fraction, heparin, natural and/or recombinant proteins from cell cultures and/or human growth factor.

7. Method according to one of the previous demands: the pharmaceutical product consists of Interleukin-2, recombinant glyco protein and/or tissue plasminogen activator.

8. Method according to one of the previous demands: as ultrafiltration membrane, an Amicon spiral membrane with tangential flow S1Y30, S10Y30 or S100Y30 is used.

9. Method according to one of the previous demands: the ultrafiltration membrane is validated through spiking with virus of the Leviviridae family.

10. Method according to one of the previous demands: control by means of a pressure test.

Table 1/2

Reduction of scrapie virus via ultrafiltration

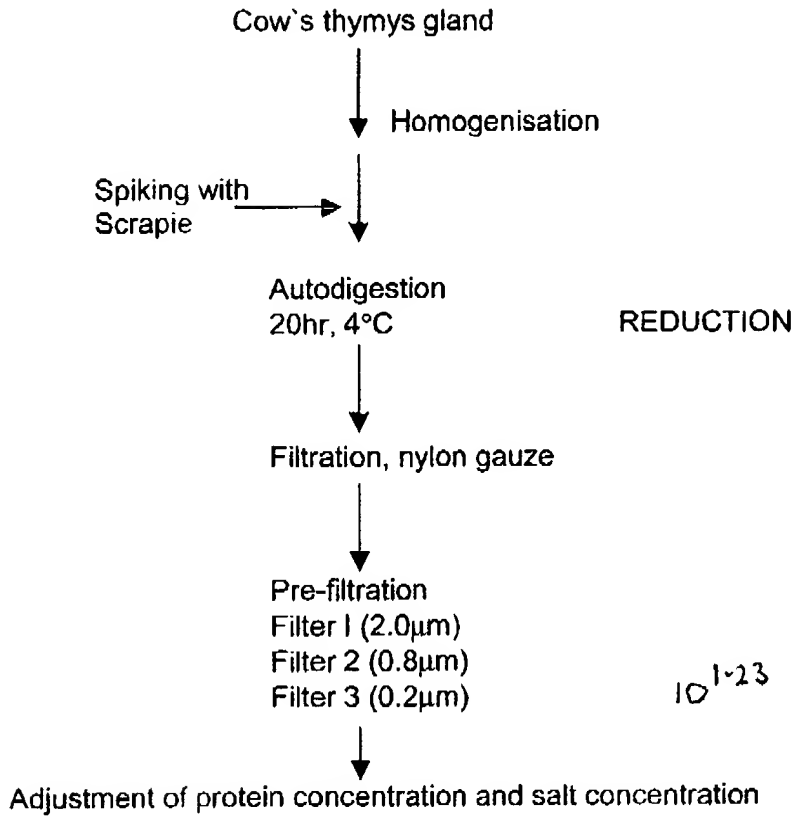


Fig 2:

Reduction of Scrapie virus
By manifold ultrafiltration

TABLE 1

REMOVAL OF PrP^{sc} BY DEPTH FILTRATION

FILTER (PRODUCT)	COMPOSITION	RETENTION (μm)	C	R
Seitz, KS80 (Albumin)	Cellulose, Kieselguhr Perlite	0.6 - 1.5	≥ 4.1	≥ 4.9
Seitz, K200P (IgG)	Cellulose, Kieselguhr Perlite	3.5 - 6.0	≥ 3.4	≥ 2.8
Cuno, Delipid 1 (Albumin)	Cellulose, Kieselguhr Cation Resin	0.6	2.8	2.3
Millipore CP20 (IgG)	Borosilicate glass	2.0	3.0	<1 *

* Large drop in PrP^{sc} measured after addition of inoculum to process feedstock.